



UNIVERSITI PUTRA MALAYSIA

**SACCHARIFICATION OF PALM OIL MILL EFFLUENT SOLID AND
OIL PALM FRUIT FIBER TO FERMENTABLE SUGARS
FOR ACETONE-BUTANOL-ETHANOL FERMENTATION**

KHAW TEIK SEONG

FSMB 2001 34

**SACCHARIFICATION OF PALM OIL MILL EFFLUENT SOLID AND OIL
PALM FRUIT FIBER TO FERMENTABLE SUGARS FOR
ACETONE-BUTANOL-ETHANOL FERMENTATION**

By

KHAW TEIK SEONG

**Thesis Submitted in Fulfilment of the Requirement for the Degree of
Master of Science in the Faculty of Food Science and Biotechnology
Universiti Putra Malaysia**

May 2001



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in the fulfilment of the requirement for the Degree of Master Science

**SACCHARIFICATION OF PALM OIL MILL EFFLUENT SOLID AND OIL
PALM FRUIT FIBER TO FERMENTABLE SUGARS FOR ACETONE-
BUTANOL-ETHANOL FERMENTATION**

By

KHAW TEIK SEONG

May 2001

Chairman : Associate Professor Dr. Arbakariya Ariff

Faculty : Food Science and Biotechnology

The effect of chemical pretreatments on saccharification of palm oil mill effluent (POME) solid and oil palm fruit fiber (OPFF) was investigated. Among the chemical pretreatments applied to the substrate (NaOH 0.5%, NH₃ 0.5%, HCl 0.5%, HNO₃ 0.5% and EDTA 0.5%), the OPFF treated with 0.5% NaOH gave the highest production of fermentable sugars. However, the saccharification performance for chemically treated POME solid was not significantly different as compared to untreated POME solid. The used of autoclaved OPFF at 121 °C, 15 psi with NaOH for 5 minute, increased the degree of hydrolysis up to 46% as compared to untreated OPFF. The optimum concentration of NaOH for the treatment of OPFF was 2%. The improvement in hydrolysis of OPFF was related to an increase of cellulose content, and a decrease in hemicellulose and lignin content.

The effect of enzyme and initial substrate concentration on the saccharification of POME solid and OPFF was investigated using two types of cellulolytic enzymes, Celluclast 1.5L (47.4 U/mL FPase, 66.0 U/mL CMCase and 51.1 U/mL β -glucosidase) and Novozyme 188 (2.79 U/mL FPase, 10.0 U/mL CMCase and 168 U/mL β -glucosidase). The highest production of reducing sugars (9.24 g/L) and glucose (4.54 g/L) from the saccharification of 5% POME solid was obtained using the Novozyme/Celluclast (N/C) ratio of 0.4. The saccharification of OPEFB using the N/C ratio of 0.25 produced 32.47 g/L total reducing sugar and 16.78 g/L glucose. The effect of initial substrate concentration on the performance of saccharification of POME solid (2% - 20% w/v) and OPFF (2% - 6%) was carried out in 2 liter stirred tank bioreactor. The highest reducing sugar (12.25 g/L) and glucose (6.70 g/L) was obtained when 15% (w/v) POME solid was used. On the other hand, the highest total reducing sugar (30.26 g/L) and glucose (16.73 g/L) was produced from 5% (w/v) OPFF.

The effect of mixing on the performance of the saccharification of CMC, POME solid and OPFF was also carried out in 2 liter stirred tank bioreactor using two different impeller diameters (48 and 84 mm). In saccharification of POME solid and OPFF, the degree of saccharification increased with increasing impeller tip speed (ITP). However, the saccharification of CMC, a soluble cellulose, increased with increasing ITP up to 2.01 m/s. Among the types of cellulosic material investigated, only the degree of saccharification for OPFF and CMC was found depended on the impeller diameter.

The feasibility of using hydrolysates from enzymatic saccharification of POME solid and OPFF for acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* P262 was studied using 250 mL modified Schott bottle cultures. The highest solvent produced was obtained in fermentation using hydrolysates treated with activated charcoal. The optimum activated charcoal concentrations required to detoxify the hydrolysates from POME solid and OPFF were 2% (w/w) and 1% (w/w), respectively. Among the carbon sources investigated, the total solvent produced from the POME solid (2.10 g/L) and OPFF (3.24 g/L) hydrolysates were higher than the other carbon sources tested (xylose, cellobiose, and POME solid). Solvent was not produced when CMC and OPFF were used as substrate with butyric acid as the main product, instead.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**SAKARIFIKASI PEPEJAL SISA AIR KILANG KELAPA SAWIT DAN SERABUT
BUAH KELAPA SAWIT UNTUK FERMENTASI
ACETONE-BUTANOL-ETHANOL**

oleh

KHAW TEIK SEONG

Mei 2001

Pengerusi : Profesor Madya Dr. Arbakariya Ariff

Faculti : Sains Makanan dan Bioteknologi

Kesan pelbagai rawatan kimia terhadap sakarifikasi pepejal sisa air kilang kelapa sawit (PSAKKS) dan serabut buah kelapa sawit (SBKS) telah disiasat. Antara rawatan kimia yang digunakan ke atas substrak (NaOH 0.5%, NH₃ 0.5%, HCl 0.5%, HNO₃ 0.5% and EDTA 0.5%), SBKS yang tertinggi. Tetapi, pencapaian sakarifikasi untuk rawatan kimia PSAKKS tidak menunjukkan perbezaan yang ketara berbanding dengan PSAKKS tanpa rawatan. Autoclave SBKS pada 121°C, 15 psi dengan NaOH selama 5 minit didapati meningkatkan darjah hidrolisis sebanyak 46%. Kepekatan optimum NaOH untuk merawat SBKS ialah 2 %. Penambahan dalam hidrolisis PSAKKS dan SBKS telah dikaitkan dengan penambahan kandungan selulose dan pengurangan kandungan hemiselulose serta lignin.

Kesan kepekatan enzim dan substrak terhadap PSAKKS dan SBKS sakarifikasi telah dikaji dengan menggunakan dua jenis enzim, Celluclast (47.4 U/mL Fpase, 66.0 U/mL CmCase and 51.1 U/mL β -glucosidase) and Novozyme 188 (2.79 U/mL Fpase, 10.0 U/mL CmCase and 168 U/mL β -glucosidase). Produksi yang optimum untuk jumlah gula penapaian (9.24 g/L) dan glukosa (4.54 g/L) dari 5% PSAKKS sakarifikasi telah diperolehi dengan menggunakan nisbah Novozyme/Celluclast bernilai 0.4. Manakala sakarifikasi ke atas SBKS dengan penggunaan nisbah N/C yang bernilai 0.25 menghasilkan 32.47 g/L jumlah gula penapaian dan 16.78 g/L glucose. Kesan kepekatan substrak ke atas pencapaian sakarifikasi PSAKKS (2% - 20%) dan SBKS (2% - 6%) telah dijalankan dalam 2 Liter reaktor jenis pengadukan. Jumlah maksimum gula penapaian (12.25 g/L) dan glucose (6.70 g/l) diperolehi apabila 15% (B/I) PSAKKS digunakan. Manakala, jumlah maksimum gula penapaian (30.26 g/L) dan glukosa (16.73 g/L) dihasilkan dari 5% (B/I) SBKS.

Kesan pencampuran ke atas pencapaian sakarifikasi CMC, PSAKKS dan SBKS juga dijalankan dalam 2 Liter reaktor jenis pengadukan dengan menggunakan dua impeller yang berlainan diameter (48 dan 84mm). Dalam sakarifikasi PSAKKS dan SBKS, darjah sakarifikasi bertambah bersamaan dengan penambahan kelajuan hujung impeller (KHI). Tetapi, sakarifikasi CMC hanya bertambah dengan penambahan KHI bawah 2.01 m/s. Di antara ketiga-tiga jenis substrak yang dikaji itu, hanya darjah sakarifikasi untuk SBKS dan CMC didapati bergantung keatas diameter impeller.

Pengajian ke atas fasibiliti penggunaan hidrolisate dari PSAKKS dan SBKS untuk produksi acetone-butanol-ethanol (ABE) oleh *Clostridium acetobutylicum* P262 telah dijalankan dalam 250 mL botol Scott yang diubahsuai. Jumlah pelarut telah diperolehi dari fermentasi ABE yang dirawat dengan arang teraktif. Kepekatan optimum arang teraktif untuk menyahtoksi terhadap hidrolisate dari PSAKKS dan SBKS ialah 2 dan 1 % masing-masing. Di antara sumber karbon yang disiasat, penghasilan jumlah pelarut dari PSAKKS dan SBKS hidrolisate didapati lebih tinggi daripada sumber karbon yang lain (xylose, sellobiose dan PSAKKS). Tetapi, tiada penghasilan pelarut didapati untuk fermentasi ABE yang menggunakan CMC dan SBKS, asid butiric merupakan produk yang utama untuk kedua-dua kes.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and deepest gratitude to my supervisor, Associate Professor Dr. Arbakariya Bin Ariff for his invaluable guidance, advise, constructive comments and encouragement during the execution of my project and preparation of this thesis. My deep appreciation is also extent to the members of my supervisory committee, Professor Dr. Mohamed Ismail Abdul Karim and Associate Professor Dr. Mohd. Ali Hassan for their guidance, valuable comments and encouragement throughout this study.

Sincere thanks are also extended due to all faculty members, staff and fellow graduate and undergraduate students of Department of Biotechnology, Faculty of Food Science and Biotechnology for their advice, help and unfailing patience throughout the course of the entire project.

Last but not least, I would like to express my utmost gratitude, indebtedness and appreciation to my grandmother, parents, brothers, sister for their understanding, caring and moral support. My deepest appreciation is recorded to my beloved one for her support, sacrifices and encouragement given during the period of this study.

I certify that an Examination Committee met on 21th May 2001 to conduct the final examination of Khaw Teik Seong, on his Master of Science thesis entitled "Saccharification of Palm Oil Mill Effluent Solid and Oil Palm Fruit Fiber to Fermentable Sugars for Acetone-Butanol-Ethanol Fermentation" in accordance with Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows.

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairman)

ARBAKARIYA ARIFF, Ph.D.
Associate Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

MOHAMMED ISMAIL ABDUL KARIM, Ph.D.
Professor
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

MOHD. ALI HASSAN, Ph.D.
Associate Professor
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)



AINI IDERIS, Ph.D.
Professor/Dean of Graduate School,
Universiti Putra Malaysia

Date: 28 MAY 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.



AINI IDERIS, Ph.D.
Professor/Dean of Graduate School,
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at UPM or other institutions.



Name: Khaw Teik Seong

Date: 26/5/2001

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL SHEETS	ix
DECLARATION FORM	x
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxi

CHAPTER

1	INTRODUCTION	1
2	ENZYMATIC SACCHARIFICATION OF LIGNOCELLULOSE	5
	2.1 The Chemical and Structural Features of Lignocelluloses	5
	2.1.1 Cellulose	5
	2.1.2 Hemicelluloses	9
	2.1.3 Lignin	10
	2.2 Effect of Structural Features of Lignocellulosic Materials on Enzymatic Hydrolysis.....	11
	2.2.1 Physical Pretreatment	13
	2.2.2 Chemical Pretreatment	15
	2.2.3 Biological Pretreatment	18
	2.3 Enzymatic Saccharification of Lignocellulose	18
	2.3.1 The Cellulase System: Properties and Mode of Action	19
	2.3.2 Mechanism of Cellulose Saccharification	21
	2.3.3 Effect of Enviroment Factors on Enzymatic Saccharification of Lignocellulose	26
	2.4 Acetone-Butanol-Ethanol Fermentation	31
	2.4.1 The Microorganism	31
	2.4.2 Application of Acetone-Butanoi-Ethanol (ABE)	32
	2.4.3 Biochemistry and Physiology of ABE Fermentation	34
	2.4.4 Acetone-Butanol-Ethanol (ABE) Fermentation Substrates	37



3	GENERAL MATERIALS AND METHODS	42
3.1	General Plan of the Experimental Work	42
3.2	Enzymes	43
3.3	Substrate for Enzymatic Saccharification	45
3.3.1	Carboxymethylcellulose	45
3.3.2	Oil Palm Empty Fruit Fiber and Palm Oil Mill Effluent	45
3.4	Microorganism and Maintenance	47
3.5	Medium Composition for Acetone-Butanol-Ethanol Fermentation	48
3.5.1	Stock Culture Medium	48
3.5.2	Culture Medium	49
3.6	General Technique for Preparation of Strict Anaerobic Medium	50
3.7	Analytical Methods	51
3.7.1	Cell Concentration Determination	51
3.7.2	Determination of Total Reducing Sugars	52
3.7.3	Determination of Glucose	52
3.7.4	Determination of Sugars	53
3.7.5	Determination of Organic Acids	54
3.7.6	Determination of Acetone-Butanol-Ethanol	54
3.7.7	Determination of Cellulose, Hemicellulose and Lignin Content..	55
3.7.8	Measurement of Cellulase Activities	58
4	THE PRETREATMENT OF PALM OIL MILL EFFLUENT (POME) SOLID AND OIL PALM FRUIT FIBER (OPFF) FOR SUBSEQUENT USE AS SUBSTRATE FOR ENZYMATIC SACCHARIFICATION	62
4.1	Introduction	62
4.2	Materials and Methods	64
4.2.1	Enzymes	64
4.2.2	Method of POME and OPFF Pretreatment	64
4.2.3	Saccharification Experiment	65
4.2.4	Analytical Procedures	66
4.3	Results and Discussion	66
4.3.1	Effect of Different Treatments on Chemical Composition of Palm Oil Mill Effluent (POME) and Oil Palm Fruit Fiber (OPFF)	66
4.3.2	Test on the Feasibility of Pretreated POME Solid and OPFF as Substrate for Enzymatic Saccharification	70
4.3.3	Effect of Chemical Pretreatment on the Saccharification of OPFF.....	74
4.3.4	The Effect of Autoclaving on Saccharification of POME solid and OPFF	76
4.3.5	Effect of Different NaOH Concentration on the Saccharification of OPFF	78

4.3.6 Relationship between Cellulose and Lignin Content on Degree of Hydrolysis from Saccharification of POME Solid and OPFF ..	80
4.4 Conclusion	82
5 OPTIMIZATION OF ENZYME AND INITIAL SUBSTRATE CONCENTRATION IN THE ENZYMATIC SACCHARIFICATION OF PALM OIL MILL EFFLUENT (POME) SOLID AND OIL PALM FRUIT FIBER (OPFF)	83
5.1 Introduction	83
5.2 Materials and Methods	85
5.2.1 Enzymes	85
5.2.2 Substrate Preparation	85
5.2.3 Saccharification Experiment	86
5.2.4 Analytical Procedures	87
5.3 Results and Discussion	87
5.3.1 Effect of Enzyme Concentration on Saccharification of POME Solid and OPFF	87
5.3.2 Effect of the Addition of β -glucosidase on Saccharification of POME Solid and OPFF	92
5.3.3 Effect of Initial Substrate Concentration on Saccharification of POME Solid and OPFF	97
5.4 Conclusion	101
6 EFFECT OF MIXING ON ENZYMATIC SACCHARIFICATION OF PALM OIL MILL EFFLUENT (POME) SOLID AND OIL PALM FRUIT FIBER (OPFF) TO FERMENTABLE SUGARS IN STIRRED TANK BIOREACTOR	102
6.1 Introduction	102
6.2 Materials and Methods	103
6.2.1 Enzymes	103
6.2.2 Substrate	104
6.2.3 Equipment	104
6.2.4 Saccharification Experiment	106
6.2.5 Analytical Procedures	107
6.2.6 Statistical Analysis	108
6.3 Results and Discussion	108
6.3.1 Effect of Impeller Tip Speed (ITP) and Diameter on the Production of Glucose from Saccharification of POME Solid, OPFF and CMC	108
6.3.2 Relationship between Impeller Tip Speed (ITP) and Impeller Diameter and Degree of Saccharification (DS)	110
6.4 Conclusion	116

7	UTILIZATION OF FERMENTATION SUGARS FROM SACCHARIFICATION OF PALM OIL MILL EFFLUENT (POME) AND OIL PALM FRUIT FIBER (OPFF) FOR ACETONE-BUTANOL- ETHANOL (ABE) FERMENTATION	117
	7.1 Introduction	117
	7.2 Materials and methods	120
	7.2.1 Microorganisms	120
	7.2.2 Fermentation Medium	120
	7.2.3 ABE Fermentation	121
	7.2.4 Analytical Procedures	121
	7.3 Results and Discussion	121
	7.3.1 Effect of Detoxification Treatment on Fermentability of Hydrolysates	121
	7.3.2 Effect of Different Activated Charcoal Concentration on Solvent Production of ABE Fermentation Using POME Solid and OPFF Hydrolysate as Substrate	124
	7.3.3 Effect of Different Carbon Sources on Solvent Fermentation	127
	7.3.4 Sugars Utilization from POME Solid and OPFF Hydrolysate by <i>C. acetobutylicum</i> P262	131
	7.4 Conclusion	134
8	GENERAL DISCUSSION, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK	136
	BIBLIOGRAPHY	140
	APPENDICES	150
	BIOGRAPHICAL SKETCH	156

LIST OF TABLES

Table		Page
1	Annual consumption and availability of biomass in the world	2
2	Chemical composition of various lignocellulosic material.....	7
3	Pretreatment method of lignocellulosic materials to enhance enzymatic hydrolysis to fermentable sugar.....	13
4	The degree of synergism between cellulase components of various cellulase producing fungus solubilization of cotton.....	23
5	Synergistic action between cellobiohydrolases I and II of <i>P. pinophilum</i> and the endoglucanases of other fungi in solubilizing cotton fiber.....	24
6	Inhibition of <i>Penicillium pusillum</i> cellulase by sugar.....	29
7	Type of raw materials used for ABE production by <i>Clostridium acetobutylicum</i>	41
8	Activity of enzyme in Celluclast and Novozyme preparations.....	43
9	Composition of Reinforced Clostridia medium.....	48
10	Composition of culture medium.....	49
11	Chemical composition of untreated and treated POME, OPFF, OPEFB and CF with different chemical and physical treatments.....	68
12	Reducing sugar and glucose production from enzymatic saccharification of POME solid and OPFF pretreated with different types chemical.....	72
13	Enzymes activities in Celluclast and Novozyme preparation.....	85
14	Sugars production from saccharification of POME solid and OPFF using different enzyme (Celluclast) concentrations.....	88

15	Reducing sugar and glucose production from saccharification of POME solid and OPFF using different enzyme (Novozyme) concentrations.....	94
16	Reducing sugar and glucose production from saccharification of POME solid and OPFF using different substrate concentrations.....	101
17	Agitation speed for the saccharification experiment using 48 and 84 mm impeller size.....	106
18	Dimension of bioreactor.....	106
19	Final glucose concentration from saccharification process using two different diameters and agitated at different impeller tip speed.....	112
20	Equation for the relationship between saccharification and impeller tip speed.....	
21	Solvent production from ABE fermentation using POME solid and OPFF hydrolysate as substrate treated with different detoxification agent.....	123
22	Solvent production from ABE fermentation using POME solid and OPFF hydrolysate as substrate treated with different concentration of activated charcoal.....	125
23	Effect of different carbon sources on cell growth and ABE production by <i>Clostridium acetobutylicum</i> P262 in defined medium.....	130
24	Sugars composition of POME solid and OPFF hydrolysate after ABE fermentation by <i>Clostridium acetobutylicum</i> P262.....	132

LIST OF FIGURE

Figure		Page
1	Structure of cellulose molecule.....	6
2	Alignment and composition of elementary fibrils of cellulose (A); Bundle of parallel elementary fibrils (hald together crosswise by hydrogen bonds, (B); Lateral sectional view of one fibril	8
3	Structural formula of acetyl-4-metylglucurono-xylan	9
4	Structural form of O-acetyl-galacto-glucomannans	10
5	Three structural monomer units lignin	11
6	Mechanism of synergistic action of CBH 1 in solubilizing cellulose	13
7	Biochemical pathways of acetone-butanol-ethanol (ABE) production by <i>Clostridium acetobutylicum</i>	39
8	Flow diagram of experimental work	44
9	Apparatus for deoxygenating medium	50
10	Time course of reducing sugar and glucose production during saccharification of POME solid pretreated with different chemical	73
11	Time course of reducing sugar and glucose production during saccharification of OPFF pretreated with different chemical	75
12	Effect of autoclaving chemically treated (A) POME and (B) OPFF on the performance of saccharification process	77
13	Time course of reducing sugar and glucose production from enzymatic saccharification of OPFF treated with different NaOH concentration ..	79
14	Relationship between cellulose and lignin contents on the degree of hydrolysis from saccharification of (A) POME and (B) OPFF	81

15	Effect of different cellulase (Celluclast) concentration on the performance of saccharification of POME solid	89
16	Effect of different cellulase (Celluclast) concentration on the performance of saccharification of OPFF	91
17	Effect of increasing amount of β -glucosidase (Novozyme) into cellulase enzyme preparation with low level of β -glucosidase (Celluclast) on the performance of saccharification of POME solid	93
18	Effect of increasing amount of β -glucosidase (Novozyme) into cellulase enzyme preparation with low level of β -glucosidase (Celluclast) on the performance of saccharification of OPFF solid	95
19	Time course of cellobiose concentration during the saccharification of POME solid using different Novozyme concentration	96
20	Time course of cellobiose concentration during the saccharification of OPFF solid using different Novozyme concentration	97
21	Profile of total reducing sugar and glucose production during enzymatic saccharification of different concentration of POME solid	99
22	Profile of total reducing sugar and glucose production during enzymatic saccharification of different concentration of OPFF solid	100
23	Schematic diagram for 1.5 L bioreactor	105
24	Profile of glucose production during enzymatic scahharification of (A) POME solid; (B) OPFF; (C) CMC using different impeller tip speed and diameter (Di)	111
25	The effect of impeller diameter on the degree of saccharification of (A) POME solid; (B) OPFF; (C) CMC	113
26	Relationship between saccharification and impeller tip speed for POME solid, OPFF and CMC	115
27	Effect of different detoxification treatment on total solvent production from ABE fermentation using POME solid hydrolysate and OPFF hydrolysate as substrate	123

28	Effect of different activated charcoal concentration on solvent production for ABE fermentation of (A) POME solid hydrolysate; (B) OPFF hydrolysate	126
29	Effect of different carbon source on ABE production	129
30	Time course of ABE fermentation by <i>Clostridium acetobutylicum</i> P262 in 250 mL modified Scotch bottle using POME solid hydrolysate as substrate	133
31	Time course of ABE fermentation by <i>Clostridium acetobutylicum</i> P262 in 250 mL modified Scotch bottle using OPFF solid hydrolysate as substrate	135

LIST OF ABBREVIATIONS

D	: Impeller Diameter (m)
N	: Impeller Rotational Speed (rps)
ITP	: Impeller Tip Speed (m/s)
ABE	: Acetone-Butanol-Ethanol
POME	: Palm Oil Mill Effluent
OPFF	: Oil Palm Fruit Fiber
CMC	: Carboxymethylcellulose
m	: Meter
L	: Litre
mL	: Mililitre
μm	: Micrometer
nm	: Nanometer
rpm	: Rotation per minute
UV	: Ultra Violet
HPLC	: High Performance Liquid Chromatography

CHAPTER 1

INTRODUCTION

The energy requirements for all activities of mankind mainly depended on fossil resources such as petroleum, natural gas and coal. Unfortunately, these fossil resources that deposited and formed over billions of years were limited in stock. Therefore, the exhaustion of these non-renewable fossil fuel stocks in the near future has prompted widespread global efforts for the development of renewable energy resources.

Biomass in the form of photogenic plants is reproduced abundantly year after year with the help of solar energy. According to recent studies, 170×10^9 tonnes of biomass are produced annually as a result of photosynthesis (Table 1). Under the legitimate assumption, biomass consists of about 40% of polysaccharides especially cellulose and starch. The annual production for these photosynthetically produced cellulose and starch are approximately 70 billion tonnes, which can be considered as high yield as compared to finite world reserves of fossil fuel. Ironically, it is only about 3% of there annually renewed biomass are being used. In other word, that is about 66 billion tonnes of these natural raw materials ends up as collectible wastes (Hans, 1993).

In Malaysia, commercial cultivation of oil palm in Malaysia was started in 1917. The pace of the development was slowed to begin with, but picked up rapidly in the 60's

and 70's (Gurmit, 1994). Today, Malaysia is the world's leading producer and exporter of palm oil. The area of palm oil plantation in 1999 was estimated at 3.31 million hectares with the production of about 10.55 million tonnes of palm oil (PORLA, 1999). As a vegetable oil seed crop, oil palm is an efficient converter of solar energy into biomass. Unfortunately, besides being a prolific producer of palm and kernel oil, its also generates a number of lignocellulosic residues and by product such as palm oil mill effluent (POME) and oil palm fruit fiber (OPFF), which are highly polluting. Although the treatment of these lignocellulosic wastes have already been established, the commercially application of these agro-industrial wastes for the production of valuable products is not yet exploited. Thus, an innovative way to treat the POME and OPFF couple with the production of valuable product should be developed.

Table 1: Annual consumption and availability of biomass in the world (in tonnes).

Biomass, annually photosynthesis yeild:	170 X 10 ⁹
Utilized by or in from of :	
Felling of trees (only major countries)	0.80 X 10 ⁹
- use for paper	0.15 X 10 ⁹
- use in chemical applications	0.007 X 10 ⁹
Cereals (all kinds)	1.45 X 10 ⁹
Natural fibers (all kinds)	0.022 X 10 ⁹
Seed products (incl. Oil seeds)	0.18 X 10 ⁹
- vegetable oils	0.05 X 10 ⁹
Potatoes	0.37 X 10 ⁹
Sugar cane and sugar beets	0.58 X 10 ⁹
Fruits (all kinds)	0.28 X 10 ⁹
Foodstuffs (of animal origin)	0.28 X 10 ⁹
Animal feed	<u>0.80 X 10⁹</u>
Total ascertainable utilization	4.969 X 10 ⁹ (approx. 2.9%)

Source: Hans (1993)

As lignocellulosic materials, POME and OPFF consist of three main chemical components; cellulose, hemicellulose and lignin. The cellulosic portions of lignocellulose are convertible into fermentable sugars, which in turn, can be used to derive polymeric materials, chemical feedstock and solvent (Ghose et al., 1979). The first step in converting lignocellulose to sugars is the saccharification process using either acid or enzyme as catalyst. However, the structural properties of lignocelluloses such as lignin-hemicellulose complex and the degree of crystallinity have made the lignocellulose recalcitrant for saccharification. Therefore, various physico-chemical pretreatments have been developed to facilitate the saccharification process over the past few decades. It is accepted that all such pretreatments were added considerably to the overall cost of saccharification. Thus, an economic viable pretreatment process for lignocellulosic material should be investigated to improve production of fermentable sugars through saccharification process.

During the first part of this century, the anaerobic production of acetone-butanol-ethanol (ABE) by solventogenic clostridia was the second largest biotechnological process in the world. This fermentation was initially aimed at the production of acetone for the war industry then the production of butanol for the lacquer industry and later the production of ethanol as biofuel which was mixed together with petrol for automobile industry (Jones and Wood, 1986). After the World War II, petroleum-based production of solvents replaced the biological processes and, as a result, almost all the industrial-scale fermentation facilities have been closed (Durre, 1998). The oil crisis in the 1970s revived interest in ABE fermentation because of the recent